

# Growth, biochemical variables, and zinc bioaccumulation in Nile tilapia, *Oreochromis niloticus* (L.) as affected by water-born zinc toxicity and exposure period

Mohsen Abdel-Tawwab · Gamal O. El-Sayed · Sherien H. Shady

Received: 26 September 2015 / Accepted: 25 May 2016 / Published online: 28 June 2016  
© The Author(s) 2016. This article is published with open access at Springerlink.com

**Abstract** The present study was carried out to investigate the effect of sublethal zinc (Zn) concentrations on growth performance, biochemical variables, and Zn residues in various organs of Nile tilapia, *Oreochromis niloticus* (L.). Fish ( $25 \pm 0.5$  g) were exposed to 0.0, 3.5, or 7.0 mg Zn L<sup>-1</sup> for 1 or 6 weeks. Fish growth was significantly reduced with increasing Zn concentrations. However, fish exposed to 7.0 mg Zn L<sup>-1</sup> grew less quickly than those of the control group. Likewise, best feed intake and feed conversion ratio were obtained at the control group. Furthermore, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and cortisol increased significantly with increasing Zn concentrations and exposure time, with maximal values in the 7.0 mg Zn L<sup>-1</sup> treatment after 6 weeks. Meanwhile, highest values of serum protein and lipids were obtained in the control fish reared for 6 week, whereas their lowest values were observed in fish exposed to 7.0 mg Zn L<sup>-1</sup> for 1 week. There was no significant change in whole-body moisture content of fish due to Zn exposure, although crude protein and total lipid contents decreased significantly with increasing Zn concentrations. In addition, Zn exposure increased total ash contents and Zn residues in different investigated fish organs. The Zn concentrations in all fish organs were time-dependant and the Zn residues after 1 week were found to follow the order of gills > liver > kidney > muscle, meanwhile after 6 weeks it followed the order of liver > kidney > gill > muscle. The present findings revealed that liver and kidney tissues are the prime sites of Zn bioaccumulation, while Zn load in the muscle was for low as compared to other organs.

**Keywords** Nile tilapia · Fish performance · Biochemical variables · Zn toxicity · Zn bioaccumulation · Fish organs

## Introduction

Heavy metals pollution is a major ecological concern due to its high persistence in the environment. The agricultural and industrial activities are the main source of heavy metal pollution, which adversely affect the aquatic ecosystem (Rashed 2001; Yilmaz 2003; Khare and Singh 2002). Although aquatic ecosystems are

---

M. Abdel-Tawwab (✉) · S. H. Shady  
Department of Fish Biology and Ecology, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt  
e-mail: mohsentawwab@gmail.com; mohsen\_tawwab@yahoo.com

G. O. El-Sayed  
Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

equipped with a variety of physico-chemical and biological mechanisms to eliminate or reduce adverse effects of toxic substances, toxicants may evoke changes in development, growth, reproduction and behavior, or may cause death of freshwater organisms (see Eisler 1993).

Zinc (Zn) is one of the most important essential trace elements involved in animal growth and the most widely used metal cofactor of many enzymes involved in protein, nucleic acid, carbohydrate, and lipid metabolism (Carpene et al. 2003; Sun et al. 2005). Zinc in certain concentration is desirable for fish growth but its over accumulation is hazardous to exposed fish (Senthil Murugan et al. 2008). Zinc is one of the most common contaminants in aquatic systems and is associated with urban runoff, soil erosion, industrial discharges, pharmaceuticals, pesticides and a variety of other activities and sources (Schmitt 2004; Bowen et al. 2006). The danger of Zn is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically and is only transformed from one oxidation state or organic complex to another (Everall et al. 1989).

Fish are an integral component of the aquatic ecosystems. In addition to being a source of protein to humans, they play important roles as bioindicators of trace element pollution (Rashed 2001). For this reason, the utility of fish for assessing environmental conditions in aquatic ecosystems has gained prominence in recent years (Yilmaz 2003; Budambula and Mwachiro 2006; Adeniyi et al. 2008; Palaniappan et al. 2010). Due to the deleterious effects of Zn on aquatic ecosystems, it is necessary to monitor its potential impact on fish performance and health.

Nile tilapia, *Oreochromis niloticus* (L.) is commonly found in a wide range of freshwater ecosystems that may be polluted by Zn. Zinc concentrations in some Egyptian lakes are ranged from 0.004 to 0.46 mg L<sup>-1</sup> (Saeed and Shaker 2008) and in some heavy-polluted lakes, Zn concentration reached 7.94 mg L<sup>-1</sup> (Abdel-Baky et al. 1998). Hence, the present study was aimed to investigate effect of water-born Zn toxicity on growth performance, biochemical variables, and whole-body chemical composition of Nile tilapia. Zinc bioaccumulation and distribution in gill, liver, muscles, and whole-fish body were also investigated.

## Materials and methods

### Experimental procedures

Healthy Nile tilapia (25 ± 0.5 g) were collected from nursery ponds, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were kept for 2 weeks in an indoor fiberglass tank for acclimation during which fish were fed a commercial diet containing 25 % crude protein (CP) up to satiation twice a day. The authors declare that this experiment followed the ethical guidance for animal research.

The metal zinc in the form of zinc sulfate (ZnSO<sub>4</sub>·7H<sub>2</sub>O-Analar grade, Merck) was used in the present study. A preliminary study was conducted to calculate the 96-h LC<sub>50</sub> of Zn for Nile tilapia according to Behrens–Karber’s method (Klassen 1991) and it was 70.0 mg L<sup>-1</sup> (Abdel-Tawwab et al. 2011). The experiment was carried out in 12 100-L glass aquaria and each aquarium was stocked with 10 fish and supplied by air via air-stone using air pump. Fish were exposed to 0.0 (control), 3.5, or 7.0 mg Zn L<sup>-1</sup> over 1 or 6 weeks where each treatment was represented by four replicates. During the experimental running, fish were fed a supplementary diet (25 % CP) up to satiation twice a day. Fish excreta with a half of the water in each aquarium were siphoned every day and replaced by dechlorinated tap-water containing the same Zn concentration. After 1 and 6 weeks of Zn post-exposure, all fish from two aquaria from each group were collected, counted, and weighed. Then, five fish per aquarium were dissected and different tissues of gill, liver, kidney, and muscles were taken separately. These tissues were washed in redistilled water and preserved at -20 °C until analysis.

### Analysis of water physico-chemical parameters

Water samples for chemical parameters were monitored weekly during the experimental period. Dissolved oxygen and temperature were measured daily on site with an oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Unionized ammonia was measured using Multiparameter Ion Analyzer (HANNA Instruments, Rhodes Island, USA). The pH value was measured using a pH-meter (Digital



Mini-pH-Meter, model 55, Fisher Scientific, Denver, USA). The electric conductivity of aquaria water was measured by a conductivity-meter (YSI model 33, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Total alkalinity and total hardness were measured by titration as described by Boyd (1984).

#### *Growth parameters and feed utilization*

Growth performance was determined and feed utilization was calculated as following:

$$\text{Weight gain} = W_2 - W_1;$$

$$\text{Specific growth rate (SGR)} = 100 [\text{Ln } W_2(\text{g}) - \text{Ln } W_1(\text{g})]/T;$$

where  $W_2$  is final weight,  $W_1$  is initial weight, and  $T$  is the experimental period (day);

Feed conversion ratio (FCR) = feed intake/weight gain.

#### Biochemical measurements

After the 1st or the 6th week post-exposure, fish were not fed during the 24 h immediately prior to blood sampling. Five fish from each aquarium were anaesthetized with buffered tricaine methane sulfonate ( $30 \text{ mg L}^{-1}$ ) and blood was collected from the caudal vasculature. The collected blood was centrifuged at  $5000 \times g$  for 15 min at room temperature. The collected serum was stored at  $-20 \text{ }^\circ\text{C}$  for further assays. Glucose total protein, total lipids, and creatinine were calorimetrically determined according to Trinder (1969), Henry (1964), Joseph et al. (1972), and Henry (1974), respectively. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in fish serum were determined colorimetrically according to Reitman and Frankel (1957). Serum cortisol levels were determined using electrochemiluminometric assay by Elecsys and Cobas e 411 Immunoassay Analyzer (Roche Diagnostics, Indianapolis, IN 46256 USA). The test kit was prepared in accordance with the method described by Chiu et al. (2003).

#### Proximate chemical analyses

The proximate chemical analyses of the whole-fish body from each treatment were carried out according to the standard methods of AOAC (Helrich 1990) for moisture, crude protein, total lipids, and total ash. Moisture content was estimated by drying the samples at  $85 \text{ }^\circ\text{C}$  in a heat oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA) for 48 h. Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 h and total ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at  $550 \text{ }^\circ\text{C}$  for 6 h.

#### Zinc residue

For measuring Zn residues in water, 1-L water sample from each aquarium was filtered via  $0.8 \text{ }\mu\text{m}$  Millipore acetylcellulose filter paper (Millipore, Bedford, MA, USA), digested with 10 ml concentrated  $\text{H}_2\text{SO}_4$  on hot plate at  $70 \text{ }^\circ\text{C}$ , concentrated to 50 ml, and transferred to a volumetric flask. Samples were adjusted up to 100 ml with redistilled water.

For measuring Zn residues in the investigated fish organs, a gram from each organ was placed in crucible and ashed in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) for 6 h. The whole-fish body was oven-dried at  $85 \text{ }^\circ\text{C}$  until constant weight and 1.0 g dry weight was ashed in a muffle furnace for 6 h. Ash was digested with 5 ml concentrated  $\text{H}_2\text{SO}_4$  and gradually kept at  $130 \text{ }^\circ\text{C}$  on hot plate until complete dryness. Then, the digests were diluted with 2 N HCl to a constant volume. The Zn concentration was determined with an atomic absorption spectrophotometer (Thermo 6600, Thermo Electron Corporation, Cambridge, UK), which was calibrated using Zn standard solutions.

## Statistical analysis

The obtained data were subjected to two-way ANOVA, to test effects of Zn concentration and exposure period as the two factors. Duncan's Multiple Range test was used as a post hoc test to compare between means at  $P \leq 0.05$ . The software SPSS, version 15 (SPSS, Richmond, Virginia, USA) was used as described by Dytham (1999).

## Results and discussion

No significant changes were observed in water temperature, dissolved oxygen, pH, EC, total alkalinity, and total hardness as a result of either increased Zn concentration, exposure time, and/or their interaction ( $P > 0.05$ ; Table 1). The concentration of unionized ammonia was increased significantly with increasing Zn concentration, from  $0.71 \text{ mg L}^{-1}$  in the control aquaria to  $0.98 \text{ mg L}^{-1}$  in the  $7.0 \text{ mg Zn L}^{-1}$  treatment after 6 weeks (Table 1), suggesting that ammonia excretion may be induced by Zn stress, e.g., as noted by Wendelaar Bonga (1997), who reported that, during stress, elevation of cortisol stimulated the production of ammonia. Similar results found increases in ammonia concentrations due to copper toxicity in common carp (De Boeck et al. 2007; Kunwar et al. 2009) and due to Zn toxicity in Nile tilapia (Abdel-Tawwab et al. 2012).

The water-born Zn exposure regimes employed in the present study were well tolerated by Nile tilapia as portrayed by the high fish survival (96.7–100.0 %). Fish performance and feed utilization, however, were significantly affected by Zn concentration, exposure time, and their interaction ( $P < 0.05$ ; Table 2). For instance, fish growth was significantly reduced with increasing Zn concentration, e.g., the fish exposed to  $7.0 \text{ mg Zn L}^{-1}$  for 6 weeks (32.2 g) grew less quickly than that the control group (44.7 g). Likewise, feed intake decreased, while FCR increased significantly with increasing Zn concentration ( $P < 0.05$ ; Table 2). The best feed intake and FCR were obtained at the control group (32.8 g feed fish<sup>-1</sup> and 1.68, respectively). One hypothesis for these observations is that exposure to elevated Zn concentrations leads to reduced fish appetite, in turn resulting in reduced feed intake and growth. An alternative hypothesis is that due to the reduced feed intake, the energy requirements were met via the decomposition of the storage-deposited nutrients (Abdel-Tawwab et al. 2006). This latter hypothesis is supported by a significant decrease in total lipids deposition observed in the current study, and consistent with Shukla and Pandey (1986), who reported significant decreases in growth of *Channa punctatus*, when exposed to  $12 \text{ mg L}^{-1}$  zinc sulfate. Also, Abdel-Tawwab et al. (2012) and (2013) found significant decreases in the growth of Nile tilapia and common carp respectively when exposed to Zn toxicity.

All the biochemical parameters monitored at 1 or 6 weeks were significantly positively or negatively affected by the Zn treatments ( $P < 0.05$ ; Table 3). For instance, glucose, AST, ALT, creatinine, and cortisol increased significantly ( $P < 0.05$ ) with increasing Zn concentration and exposure time, with maximal values of glucose, AST, ALT, creatinine, and cortisol observed in the  $7.0 \text{ mg Zn L}^{-1}$  treatment after 6 weeks ( $143.8 \text{ mg dL}^{-1}$ ,  $81.4$ ,  $59.2 \text{ IU dL}^{-1}$ ,  $0.79 \text{ mg dL}^{-1}$ , and  $9.39 \text{ } \mu\text{g dL}^{-1}$ , respectively; Table 3). On the other hand, serum protein and lipid concentrations decreased significantly with increasing Zn concentration. Highest values of serum protein and lipids ( $7.93$  and  $6.85 \text{ g dL}^{-1}$ , respectively) were obtained in the control fish reared for 6 week, whereas lowest values were observed in fish exposed to  $7.0 \text{ mg Zn L}^{-1}$  for 1 week ( $1.80$  and  $2.31 \text{ g dL}^{-1}$ , respectively; Table 3). The increased blood glucose and cortisol concentrations due to Zn exposure suggests that the Zn caused stress. The primary response against stress involves the increases in plasma cortisol (Barton and Iwama 1991; Barton 2002). This hormone induces secondary stress responses, characterized by increased glucose levels, mobilizing glucose to tissues for homeostasis to cope with energy demanding processes of restoration (Wendelaar Bonga 1997; Barton et al. 2002). Stress may thus have resulted in a high consumption of energy reserves, and this reallocation of metabolic energy may have negatively interfered with other physiological processes, viz. growth, reproduction, and immunity etc. (Barton and Iwama 1991; Wendelaar Bonga 1997; Pickering 1998; Mommsen et al. 1999).

ALT and AST activities are frequently used in the diagnosis of damage caused by pollutants in liver tissues (Coppo et al. 2003; Chen et al. 2004). The increased AST and ALT activities observed in this study may be indicative of liver damage, which in turn may have led to the leakage of these enzymes from liver cytosol into the blood stream. This is consistent with Firat and Kargin (2010) who found increases in ALT and AST activity in Nile tilapia serum caused by the individual and combined effects of exposure to Zn and Cd. Abdel-Tawwab et al. (2012) and (2013) found significant increases in ALT and AST activity in Nile tilapia and common carp, respectively, when exposed to different Zn concentrations.



**Table 1** Changes in aquaria's water physico-chemical parameters (mean  $\pm$  SE) stocked by Nile tilapia exposed to different water-born Zn concentrations for different periods

Zn concentrations (mg L <sup>-1</sup> )	Temperature (°C)	Dissolved O <sub>2</sub> (mg/L)	pH	Conductivity ( $\mu$ S/cm)	NH <sub>3</sub> (mg/L)	Total alkalinity (mg/L)	Total hardness (mg/L)	Water-borne Zn (mg/L)
After 1 week								
0.0	24.9 $\pm$ 0.13	4.9 $\pm$ 0.4	7.52 $\pm$ 0.06	422.3 $\pm$ 1.7	0.71 $\pm$ 0.21 c	193.3 $\pm$ 6.7	228.0 $\pm$ 6.1	0.43 $\pm$ 0.12
3.5	25.0 $\pm$ 0.11	4.8 $\pm$ 0.3	7.46 $\pm$ 0.02	421.0 $\pm$ 4.1	0.82 $\pm$ 0.15 bc	200.0 $\pm$ 5.0	231.3 $\pm$ 5.9	3.63 $\pm$ 0.09
7.0	24.8 $\pm$ 0.13	4.9 $\pm$ 0.3	7.48 $\pm$ 0.04	420.9 $\pm$ 3.1	0.88 $\pm$ 0.18 b	191.7 $\pm$ 6.0	230.0 $\pm$ 8.1	7.72 $\pm$ 0.16
After 6 weeks								
0.0	24.4 $\pm$ 0.31	5.3 $\pm$ 0.2	7.52 $\pm$ 0.06	415.2 $\pm$ 2.6	0.79 $\pm$ 0.08 c	190.0 $\pm$ 7.6	231.3 $\pm$ 5.9	0.41 $\pm$ 0.14
3.5	24.5 $\pm$ 0.23	5.2 $\pm$ 0.3	7.46 $\pm$ 0.07	420.2 $\pm$ 2.6	0.85 $\pm$ 0.19 b	196.7 $\pm$ 7.3	228.0 $\pm$ 6.1	3.59 $\pm$ 0.18
7.0	24.6 $\pm$ 0.18	4.9 $\pm$ 0.3	7.41 $\pm$ 0.03	421.9 $\pm$ 2.5	0.98 $\pm$ 0.10 a	190.0 $\pm$ 7.6	236.3 $\pm$ 8.2	7.63 $\pm$ 0.18
Two-way ANOVA								
Zn concentration	0.717	0.729	0.224	0.649	0.013	0.298	0.840	0.001
Exposure period (EP)	0.707	0.320	0.547	0.349	0.044	0.067	0.711	0.667
Zn conc. $\times$ EP	0.900	0.834	0.687	0.368	0.960	0.130	0.772	0.886

Means having the same letter in the same column are not significantly different at  $P < 0.05$ 

**Table 2** Growth performance (mean  $\pm$  SE) of Nile tilapia exposed to different water-born Zn concentrations for different periods

Zn concentrations (mg L <sup>-1</sup> )	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (%/day)	Feed intake (g feed/fish)	FCR	Survival (%)
After 1 week							
0.0	25.3 $\pm$ 0.07	29.5 $\pm$ 0.76 d	4.3 $\pm$ 0.81 d	2.250 $\pm$ 0.395 a	4.1 $\pm$ 0.03 d	0.95 $\pm$ 0.23 d	100.0 $\pm$ 0.0
3.5	25.2 $\pm$ 0.07	27.6 $\pm$ 0.15 de	2.4 $\pm$ 0.17 de	1.300 $\pm$ 0.092 b	3.2 $\pm$ 0.14 de	1.33 $\pm$ 0.16 c	96.7 $\pm$ 3.3
7.0	25.3 $\pm$ 0.06	26.1 $\pm$ 0.17 d	1.0 $\pm$ 0.08 e	0.558 $\pm$ 0.043 d	2.8 $\pm$ 0.32 e	2.80 $\pm$ 0.47 b	96.7 $\pm$ 3.3
After 6 weeks							
0.0	25.2 $\pm$ 0.09	44.7 $\pm$ 0.86 a	19.5 $\pm$ 0.89 a	1.365 $\pm$ 0.049 b	32.8 $\pm$ 0.55 a	1.68 $\pm$ 0.06 c	100.0 $\pm$ 0.0
3.5	25.3 $\pm$ 0.03	37.5 $\pm$ 1.00 b	12.3 $\pm$ 1.03 b	0.946 $\pm$ 0.066 c	25.6 $\pm$ 0.52 b	2.08 $\pm$ 0.17 b	96.6 $\pm$ 3.3
7.0	25.2 $\pm$ 0.07	32.2 $\pm$ 0.15 c	7.1 $\pm$ 0.22 c	0.593 $\pm$ 0.016 d	23.0 $\pm$ 0.12 c	3.24 $\pm$ 0.11 a	96.6 $\pm$ 3.3
Two-way ANOVA	<i>P</i> value						
Zn concentration	0.958	0.001	0.001	0.001	0.001	0.001	0.075
Exposure period (EP)	0.684	0.001	0.001	0.022	0.001	0.048	0.094
Zn conc. $\times$ EP	0.743	0.001	0.001	0.042	0.001	0.003	0.821

Means having the same letter in the same column are not significantly different at  $P < 0.05$



**Table 3** Changes in biochemical variables (mean  $\pm$  SE) of Nile tilapia exposed to different water-born Zn concentrations for different periods

Zn concentrations (mg L <sup>-1</sup> )	Glucose (mg/dL)	Protein (g/dL)	Lipids (g/dL)	AST (IU/dL)	ALT (IU/dL)	Creatinine (mg/dL)	Cortisol ( $\mu$ g/dL)
After 1 week							
0.0	82.8 $\pm$ 3.9 d	2.94 $\pm$ 0.156 c	4.07 $\pm$ 0.264 b	17.10 $\pm$ 1.159 e	23.03 $\pm$ 1.084 d	0.21 $\pm$ 0.017 d	0.89 $\pm$ 0.102 e
3.5	88.3 $\pm$ 2.2 d	1.99 $\pm$ 0.052 d	2.59 $\pm$ 0.121 c	34.00 $\pm$ 0.854 d	28.20 $\pm$ 2.117 d	0.32 $\pm$ 0.023 c	1.46 $\pm$ 0.171 de
7.0	111.3 $\pm$ 2.8 c	1.80 $\pm$ 0.088 d	2.31 $\pm$ 0.168 c	41.20 $\pm$ 1.332 cd	35.00 $\pm$ 0.577 c	0.51 $\pm$ 0.041 b	2.56 $\pm$ 0.169 c
After 6 weeks							
0.0	83.0 $\pm$ 2.5 d	7.93 $\pm$ 0.267 a	6.85 $\pm$ 0.541 a	38.33 $\pm$ 1.378 c	40.80 $\pm$ 3.859 c	0.34 $\pm$ 0.058 c	2.21 $\pm$ 0.159 cd
3.5	130.3 $\pm$ 2.1 b	5.80 $\pm$ 0.577 b	4.79 $\pm$ 0.258 b	60.70 $\pm$ 1.909 b	51.77 $\pm$ 2.113 b	0.48 $\pm$ 0.015 b	4.66 $\pm$ 0.433 b
7.0	143.8 $\pm$ 3.4 a	2.93 $\pm$ 0.353 c	4.37 $\pm$ 0.231 b	81.37 $\pm$ 4.446 a	59.20 $\pm$ 1.607 a	0.79 $\pm$ 0.049 a	9.39 $\pm$ 0.486 a
Two-way ANOVA							
Zn concentration	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Exposure period (EP)	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Zn conc. $\times$ EP	0.001	0.001	0.036	0.026	0.003	0.045	0.001

Means having the same letter in the same column are not significantly different at  $P < 0.05$ 

**Table 4** Proximate chemical analysis (mean  $\pm$  SE) (%; dry matter basis) of whole-body of Nile tilapia exposed to different water-born Zn concentrations for 6 weeks

Zn concentrations (mg L <sup>-1</sup> )	Moisture	Crude protein	Total lipids	Total ash
0.0	71.9 $\pm$ 1.81	65.6 $\pm$ 1.78 a	14.8 $\pm$ 0.51 a	18.4 $\pm$ 0.47 c
3.5	72.3 $\pm$ 1.49	63.8 $\pm$ 1.35 b	12.8 $\pm$ 0.23 b	21.6 $\pm$ 0.56 b
7.0	73.8 $\pm$ 1.21	62.7 $\pm$ 1.01 b	12.4 $\pm$ 0.91 b	24.3 $\pm$ 0.83 a

Means having the same letter in the same column are not significantly different at  $P < 0.05$

**Table 5** Changes in Zn residues (mean  $\pm$  SE) ( $\mu$ g/g fresh weight) in different organs and whole-body of Nile tilapia exposed to different water-born Zn concentrations for different periods

Zn concentrations (mg L <sup>-1</sup> )	Gills	Liver	kidney	Muscles	Whole body
After 1 week					
0.0	22.8 $\pm$ 0.74 d	24.9 $\pm$ 0.86 e	24.6 $\pm$ 0.56 e	9.5 $\pm$ 0.39 c	96.8 $\pm$ 3.93 e
3.5	42.3 $\pm$ 1.43 c	37.2 $\pm$ 0.96 d	31.3 $\pm$ 1.01 d	11.7 $\pm$ 0.67 b	178.3 $\pm$ 5.91 d
7.0	59.3 $\pm$ 1.26 b	48.4 $\pm$ 1.37 c	40.9 $\pm$ 0.98 c	12.6 $\pm$ 0.72 b	234.5 $\pm$ 4.66 c
After 6 weeks					
0.0	23.6 $\pm$ 0.38 d	25.4 $\pm$ 0.47 e	25.4 $\pm$ 0.52 e	10.5 $\pm$ 0.28 bc	98.8 $\pm$ 4.96 e
3.5	56.6 $\pm$ 0.89 b	78.5 $\pm$ 2.51 b	66.4 $\pm$ 1.57 b	19.4 $\pm$ 1.24 a	265.8 $\pm$ 7.01 b
7.0	83.2 $\pm$ 2.94 a	109.5 $\pm$ 4.36 a	93.5 $\pm$ 1.71 a	20.2 $\pm$ 0.83 a	346.7 $\pm$ 8.67 a
Two-way ANOVA					
Zn concentration	0.001	0.001	0.001	0.001	0.001
Exposure period (EP)	0.001	0.001	0.001	0.001	0.001
Zn conc. $\times$ EP	0.002	0.001	0.001	0.001	0.001

Means having the same letter in the same column are not significantly different at  $P < 0.05$

Serum creatinine is a traditional screening index for kidney function and renal structural integrity. The increased creatinine indicates that Zn toxicity had a marked effect on kidney function, perhaps due to the action of water-born Zn on glomeruli filtration rate and/or pathological changes to the kidney resulting in dysfunction. Similar results were obtained by Abdel-Tawwab et al. (2012) and (2013) who found creatinine increases in Nile tilapia and common carp, respectively, due to Zn toxicity.

There was no significant change in whole-body moisture content of fish due to Zn exposure, although crude protein and total lipid contents decreased significantly with increasing Zn concentration ( $P < 0.05$ ; Table 4). These observations may be due to the breakdown of those molecules as energetic substrates to cope with Zn-induced stress metabolically (Vijayan et al. 1997). The low proteins and lipids in Zn-exposed fish may be due to the reduced feed intake. Moreover, the loss of protein and lipid levels in the Zn-exposed fish may be due to increased protein oxidation with Zn exposure (Cakmak et al. 2006). Palaniappan et al. (2010) reported that Zn exposure caused important structural alteration in the existing proteins indicated by a significant reduction in the intensities of the  $\alpha$ -helix. They also suggested that Zn exposure causes significant alteration in the protein secondary structure by decreasing the  $\alpha$ -helix and increasing the  $\beta$ -sheet content of the gill tissues of rohiya carp, *Labeo rohita*. Due to the low feed intake by Zn-exposed fish, the deposited protein and lipid decreased and vice versa. Furthermore, changes in protein and lipid contents in fish body may be linked with changes in their synthesis and/or deposition rate in fish body (Fauconneau 1985; Abdel-Tawwab et al. 2006), or because fish exerted more energy to challenge the Zn toxicity effect. Similar results were obtained by Mohanty et al. (2009) who concluded that Zn accumulation in the whole body of Indian major carp increased with increasing Zn concentration. Abdel-Tawwab et al. (2012) and (2013) found that Zn accumulations in the whole bodies of Nile tilapia and common carp are correlated with Zn concentrations.

The concentration of the whole-body ash and Zn residue in the whole-fish body and different organs were perhaps unsurprisingly significantly affected by Zn concentration, exposure time, and their interaction ( $P < 0.05$ ; Table 5). For instance, Zn residues in the control fish reared for 1 week had lowest tissue concentrations (22.8, 24.9, 24.6, 9.5, and 96.8  $\mu$ g g<sup>-1</sup> wet weight for gills, liver, kidney, muscles, and whole body,





respectively; Table 5). Fish exposed to  $7.0 \text{ mg Zn L}^{-1}$  over 6 weeks accumulated more Zn residue than other treatments (83.2, 109.5, 95.5, 20.2, and  $346.7 \mu\text{g g}^{-1}$  wet weight in gills, liver, kidney, muscles, and whole body, respectively). The muscle tissue was always contained a significantly lower Zn than the other tissues ( $P < 0.05$ ). This is consistent with Ortiz et al. (1999), Senthil Murugan et al. (2008), and Palaniappan et al. (2010) who reported similar trends in the *Sole Senegalensis*, *Channa punctatus*, and rohita carp, respectively.

The differences in the level of accumulation in the different fish organs are primarily attributed to the differences in the physiological role of each organ (Karuppasamy 2004). For instance, upon exposure initially the gills tissues accumulated highest Zn levels because the gills play a significant role in metal uptake, storage, and eventually transfer to the internal compartments via blood transport (Romanenko et al. 1986). On prolonged exposure, Zn concentrations in liver tissues rise; an observation ascribed to the binding of Zn to hepatic metallothionein (Kendrick et al. 1992; Atli and Canli 2003). High Zn concentration in the kidneys of exposed fish may be related to the role it plays in excretion. Muscle tissues accumulated least Zn residues because they do not come in direct contact with toxicants, nor the muscle is an active site of detoxification.

## Conclusion

The present study revealed that Zn exposure had a deteriorate effect on growth performance and health of Nile tilapia. However, liver and kidney tissues are the prime sites of Zn bioaccumulation, while Zn load in muscles tissues was low as compared to other organs.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## References

- Abdel-Baky TE, Hagraas AE, Hassan SH, Zyadah MA (1998) Environmental impact assessment of pollution in Lake Manzala. I- Distribution of some heavy metals in water and sediment. *J Egypt Germ Soc Zool* 26(B):25–38
- Abdel-Tawwab M, Khattab YAE, Ahmad MH, Shalaby AME (2006) Compensatory growth, feed utilization, whole body composition and hematological changes in starved juvenile Nile tilapia, *Oreochromis niloticus* (L.). *J Appl Aquacult* 18:17–36
- Abdel-Tawwab M, El-Sayed GO, Shady SH (2011) Acute toxicity of water-born zinc in Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Proceedings of the ninth International Symposium on Tilapia in Aquaculture, Liu Liping and Kevin Fitzsimmons (eds), Shanghai Ocean University, Shanghai, China 22–24 April 2011, pp 39–44
- Abdel-Tawwab M, El-Sayed GO, Shady SH (2012) Effects of dietary protein levels and environmental zinc exposure on the growth, feed utilization, and biochemical variables of Nile tilapia, *Oreochromis niloticus* (L.). *Toxicol Environ Chem* 94(7):1368–1382
- Abdel-Tawwab, M., Mousaad, M.N.M., Sharafeldin, K.M., and Ismaiel, N.E.M. (2013). Changes in growth and biochemical status of common carp, *Cyprinus carpio* L. exposed to water-born zinc toxicity for different periods. *International Aquatic Research*, 5(11):1–9
- Adeniyi AA, Yusuf KA, Okedeyi OO (2008) Assessment of the exposure of two fish species to metals pollution in the Ogun river catchments, Ketu, Lagos, Nigeria. *Environ Monit Assess* 137:451–458
- Atli G, Canli M (2003) Natural occurrence of metallothionein-like proteins in the liver of fish *Oreochromis niloticus* and effects of cadmium, lead, copper, zinc, and iron exposures on their profiles. *Bull Environ Contam Toxicol* 70:619–627
- Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integ Comp Biol* 42:517–525
- Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Ann Rev Fish Dis* 10:3–26
- Barton BA, Morgan JD, Vijayan MM (2002) Physiological and condition-related indicators of environmental stress on fish. In: Adams SM (ed) *Biological Indicators of Aquatic Ecosystems Stress*. American Fisheries Society, Bethesda, pp 111–148
- Bowen L, Werner I, Johnson ML (2006) Physiological and behavioral effects of zinc and temperature on coho salmon (*Oncorhynchus kisutch*). *Hydrobiol* 559:161–168
- Boyd CE (1984) *Water Quality in Warm water Fishponds*. Auburn University Agriculture Experimental Station, Auburn
- Budambula NLM, Mwachiro EC (2006) Metal status of Nairobi river waters and their bioaccumulation in *Labeo cylindricus*. *Water Air Soil Poll* 169:275–291
- Cakmak G, Dogan I, Severcan F (2006) 17 $\beta$ -Estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FT-IR spectroscopy: a comparative study with nonylphenol. *Aquat Toxicol* 77:53–63



- Carpene E, Andreani G, Monari M, Kindt M, Isani G (2003) Biochemical changes during post-larval growth in white muscle of Gilthead Sea Bream (*Sparus aurata*) fed Zinc fortified diets. *Vet Res Commun* 27:215–218
- Chen CY, Wooster GA, Bowser PR (2004) Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin, or copper sulfate. *Aquaculture* 239:421–443
- Chiu SK, Collier CP, Clark AF, Wynn-Edwards KE (2003) Salivary cortisol on ROCHE Elecsys immunoassay system: pilot biological variation studies. *Clin Biochem* 36:211–214
- Coppo JA, Mussart NB, Fioranelli SA (2003) Physiological variation of enzymatic activities in blood of bullfrog, *Rana catesbeiana* (Shaw, 1802). *Rev Veter* 12:22–27
- De Boeck G, Van derVen K, Meeus W, Blust R (2007) Sublethal copper exposure induces respiratory stress in common and gibel carp but not in rainbow trout. *Comp Biochem Physiol (C)* 144:380–390
- Dytham C (1999) Choosing and using statistics: A Biologist's guide. Blackwell Science Ltd., London
- Eisler R (1993) Zinc Hazards to Fish, Wildlife, and Invertebrates: a synoptic review, biological report fish and wildlife service, vol 10. US Department of Interior, Washington DC
- Everall NC, MacFarlane NAA, Sedgwick RW (1989) The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout, *Salmo trutta* L. *J Fish Biol* 35:27–36
- Fauconneau B (1985) Protein synthesis and protein deposition in fish. In: Cowey CB, Mackie AM, Bell JG (eds) Nutrition and Feeding in Fish. Academic Press, London, pp 17–45
- Firat O, Kargin F (2010) Individual and combined effects of heavy metals on serum biochemistry of Nile tilapia *Oreochromis niloticus*. *Arch Environ Contam Toxicol* 58:151–157
- Helrich K (1990) Official Methods of Analysis, 15th edn. Association of Official Analytical Chemist Inc., Virginia
- Henry RJ (1964) Colorimetric determination of total protein. In: Clinical Chemistry. Harper and Row Publication, New York
- Henry RJ (1974) Clinical Chemistry Principles and Techniques, 2nd edn. Harper and Row Publication, New York
- Joseph A, Knight M, Anderson S, James M, Rawie H (1972) Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipid. *Clin Chem* 18(3):198–201
- Karuppasamy R (2004) Evaluation of Hg concentration in the tissue of fish *Channa punctatus* (Bloch.) in relation to short and long-term exposure to phenyl mercuric acetate. *J Plat Jubilee AU* 40:197
- Kendrick MH, May MT, Plishka MJ, Robinson KD (1992) Metals in biological system. Ellis Horwood Ltd., England
- Khare S, Singh S (2002) Histopathological lessons induced by copper sulphate and lead nitrate in the gills of fresh water fish *Nandus*. *J Ecotoxicol Environ Monit* 12:105–111
- Klassen CD (1991) Principles of toxicology. In: Gilman AG, Tall TW, Nies AS, Taylor P (eds) Pharmacological Basis of Therapeutics, 8th edn. McGraw-Hill, Berlin, pp 49–61
- Kunwar PS, Tudorache C, Eyckmans M, Blust R, De Boeck G (2009) Influence of food ration, copper exposure and exercise on the energy metabolism of common carp (*Cyprinus carpio*). *Comp Biochem Physiol (C)* 149:113–119
- Mohanty M, Adhikari S, Mohanty P, Sarangi N (2009) Effect of Waterborne Zinc on Survival, Growth, and Feed Intake of Indian Major Carp, *Cirrhinus mrigala* (Hamilton). *Water Air Soil Poll* 201:3–7
- Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Rev Fish Biol Fish* 9:211–268
- Ortiz JB, Arellano JM, Desilva C (1999) Levels of copper, zinc, manganese and iron of candiz bay. *Bull Inst Esp Oceanogr* 15:14
- Palaniappan PLRM, Nishanth T, Renju VB (2010) Bioconcentration of zinc and its effect on the biochemical constituents of the gill tissues of *Labeo rohita*: an FT-IR study. *Inf Phys Technol* 53:103–111
- Pickering AD (1998) Stress responses of farmed fish. In: Black K, Pickering A (eds) Biology of farmed fish. Sheffield Academic Press, Sheffield, pp 222–382
- Rashed MN (2001) Monitoring of environmental heavy metals in fish from Nasser Lake. *Environ Int* 27:27–33
- Reitman S, Frankel S (1957) Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Path* 28:53–56
- Romanenko VD, Malyzheva TD, Yevtushenko N (1986) The role of various organs in regulating zinc metabolism in fish. *Hydrobiol J* 21:7–12
- Saeed SM, Shaker IM (2008) Assessment of heavy metals pollution in water and sediments and their effect on *Oreochromis niloticus* in the northern delta lakes, Egypt. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, H. Elghoubashy, K. Fitzsimmons, and A.S. Diab (Editors), Cairo International Convention Center, Cairo, Egypt, 12–14 October 2008, pp 475–490
- Schmitt CJ (2004) Concentrations of arsenic, cadmium, copper, lead, selenium, and zinc in fish from the Mississippi river basin, 1995. *Environ Monit Assess* 90:289–321
- Senthil Murugan S, Karuppasamy R, Poongodi K, Puvaneswari S (2008) Bioaccumulation pattern of zinc in freshwater fish *Channa punctatus* (Bloch.) after chronic exposure. *Turk J Fish Aquat Sci* 8:55–59
- Shukla JP, Pandey K (1986) Effect of a sub-lethal concentration of zinc sulphate on the growth rate of fingerlings of *Channa punctatus* (Bloch), a freshwater murrel. *Acta Hydrochim Hydrobiol* 14:677–680
- Sun JY, Jing MY, Wang JF, Zi NT, Fu LJ, Lu MQ, Pan L (2005) Effect of zinc on biochemical parameters and changes in related gene expression assessed by cDNA micro arrays in pituitary of growing rats. *Nut* 22:187–196
- Trinder P (1969) Determination of glucose concentration in the blood. *Ann Clin Biochem* 6:24
- Vijayan MM, Cristina Pereira E, Grau G, Iwama GK (1997) Metabolic responses associated with confinement stress in tilapia: the role of cortisol. *Comp Biochem Physiol (C)* 116:89–95
- Wendelaar Bonga SE (1997) The stress response in fish. *Physiol Rev* 7:591–625
- Yilmaz AB (2003) Levels of heavy metals (Fe, Cu, Ni, Cr, Pb and Zn) in tissue of *Mugil cephalus* and *Trachurus mediterraneus* from Iskenderun Bay, Turkey. *Environ Res* 92:277–281

